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α_2-Adrenoceptor antagonist potencies of two hydroxylated metabolites of yohimbine

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1 The α_2-adrenoceptor antagonist capacities of two hydroxylated metabolites of yohimbine in man (10-OH-yohimbine and 11-OH-yohimbine) were investigated on the α_2-adrenoceptors of human platelets and adipocytes and compared to those of yohimbine.

2 Yohimbine and 11-OH-yohimbine exhibited similar α_2-adrenoceptor affinity in biological studies i.e. inhibition of adrenaline-induced platelet aggregation and inhibition of UK14304-induced antilipolysis in adipocytes.

3 Yohimbine and the two metabolites displaced [^{3}H]-RX 821002 binding with equivalent affinities in platelet and adipocyte membranes with the following order of potency: yohimbine > 11-OH-yohimbine > 10-OH-yohimbine. However, when binding studies were carried out in binding buffer supplemented with 5% albumin, the apparent affinity of yohimbine was reduced about 10 fold and was similar to that of 11-OH-yohimbine.

4 Yohimbine and its metabolites were bound to different extents to plasma proteins, the bound fraction being 82%, 43% and 32% respectively for yohimbine, 11-OH-yohimbine and 10-OH-yohimbine.

5 These results show that the main hydroxylated metabolite of yohimbine in man (11-OH-yohimbine) possesses α_2-adrenoceptor antagonist properties. The discrepancies found in binding studies (i.e. 10 fold lower affinity of 11-OH-yohimbine than yohimbine for α_2-adrenoceptors but similar capacities in blocking biological α_2-adrenoceptor effects in cells) are attributable to the higher degree of binding of yohimbine to plasma protein.

Keywords: Yohimbine: human hydroxylated yohimbine metabolites; α_2-adrenoceptors; adipocyte; platelet

Introduction

Yohimbine is an indole alkaloid obtained from a variety of botanical sources. It is a selective α_2-adrenoceptor antagonist and has been a reference for differentiating α-adrenoceptor subtypes (Goldberg & Robertson, 1983). Due to the widespread locations of α-adrenoceptors in the central and peripheral nervous system (at the pre- or post-synaptic level) yohimbine has a variety of autonomic and psychic effects related to an increase in noradrenaline release. These are presumed to result from a blockade of the presynaptic α_2-adrenoceptors (Peskind *et al.*, 1989; Grunhaus *et al.*, 1989). Various endocrine and metabolic effects observed after yohimbine administration can be due to a direct inhibition of α_2-adrenoceptors or to an effect of the released noradrenaline on other adrenoceptors located on target tissues i.e. β- or α_1-adrenoceptors (Goldberg *et al.*, 1983; Goldberg & Robertson, 1983; Galitzky *et al.*, 1988; Nichols & Ruffolo, 1991).

Yohimbine could be a useful agent in the management of autonomic failure (Onrot *et al.*, 1987) and orthostatic hypotension due to tricyclic antidepressant administration. In combination with desipramine, yohimbine has been claimed to be of value in the treatment of refractory depression (Charney *et al.*, 1986). Recently, yohimbine was shown to induce lipid mobilization in obese subjects, related to activation of the adrenergic nervous system (Berlan *et al.*, 1991) and to enhance saliva secretion in patients with dry mouth after antidepressant therapy (Rispail *et al.*, 1990).

Pharmacokinetic studies carried out in man indicate that yohimbine is rapidly eliminated from the plasma ($t_{\frac{1}{2}}$ of 0.6 h), has a low renal excretion (about 1%), a high plasma clearance, and that its bioavailability after oral administration is low (about 20–30%) and variable (Owen *et al.*, 1987; Guthrie *et al.*, 1990). These data suggested that the low bioavailability of yohimbine may result from a variable first-pass effect. Moreover, in human use, the relatively long-lasting pharmacodynamic effects of yohimbine are inconsistent with its short apparent plasma half-life (Charney *et al.*, 1986; Berlin *et al.*, 1989; Berlan *et al.*, 1991).

Recently, the existence of two hydroxylated metabolites of yohimbine has been demonstrated in human subjects. They were identified as being hydroxylated in the C-10 and the C-11 positions (Le Verge *et al.*, 1992). They were extracted from plasma, urine and detected by a fluorimetric method and then identified by mass spectral and n.m.r. spectra. This new bioavailability study demonstrated that the 11-OH-yohimbine metabolite was largely present in the plasma and exhibited a longer elimination half-life than the parent drug (6 h compared with 1 h).

The present study was conducted in order to evaluate the properties of the recently synthesized hydroxylated metabolites of yohimbine (Levy *et al.*, personal communication) on human α_2-adrenoceptors from platelets and subcutaneous adipose tissue. The binding capacities of the two hydroxylated metabolites to human platelet and adipocyte membrane α_2-adrenoceptors were investigated. The efficacy of 11-OH-yohimbine in inhibiting adrenaline-induced platelet aggregation and the antilipolytic effect of the α_2-adrenoceptor agonist, UK 14304, on isolated adipocytes is also described.

Methods

Adipocyte preparation and lipolysis measurement

Human adipose tissue was obtained from 4 patients, submitted to dermolipectomia, who had been fasted overnight

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before tissue sampling. Fat biopsies (20–30 g) were taken immediately after the induction of general anaesthesia by pentobarbitone.

Isolated adipocytes were obtained as previously described (Mauriege *et al.*, 1987) by collagenase digestion of adipose fragments in Krebs Ringer bicarbonate buffer containing albumin (3.5 g 100 ml^{-1}) (KRB) and glucose (6 mM) at pH 7.4 and 37°C with gentle shaking at around 60 cycles min^{-1}. At the end of the incubation, the adipocytes were filtered through a silk screen and washed three times with KRB buffer to eliminate collagenase. Isolated adipocytes were incubated in 1 ml KRB (pH 7.4) containing glucose (6 mM) at 37°C in polyethylene tubes under a 95% O_2/5% CO_2 gas phase with gentle shaking (60 cycles min^{-1}) in a water bath. Pharmacological agents at suitable dilutions were added to the cell suspension just before the beginning of the assay. After 90 min of incubation, the tubes were placed in an ice bath and 200 µl aliquots of the infranatant were taken for enzymatic determination of glycerol (Wieland, 1957) released in the incubation medium which was used as the index of fat-cell lipolysis.

Platelet preparation and aggregation studies

Blood was taken by antecubital venopuncture from 4 healthy male volunteers. The platelet membranes were prepared as follows: 20–30 ml of blood was collected over 2 ml of 0.16 mM sodium citrate solution and immediately centrifuged at 160 *g* for 10 min at room temperature. The platelet rich plasma (PRP) was collected and immediately used for aggregation tests.

The platelet aggregation technique was described previously (Villeneuve *et al.*, 1985). Briefly, 10 ml of venous blood from 3 healthy male subjects were collected over 2 ml of 0.16 mM sodium citrate solution and immediately centrifuged at 160 *g* for 10 min at room temperature to obtain platelet rich plasma (PRP). The platelet number was adjusted to 350,000–400,000 mm^{-3} with autologous platelet-poor plasma obtained after centrifugation (10,000 *g* for 5 min of the residual blood collected after the first centrifugation) and platelet aggregatory responses to adrenaline (6 µM) were examined by a turbidimetric method using a Coultronic apparatus. The effect of increasing concentrations of yohimbine or 11-OH-yohimbine (0.1, 1 and 10 µM) was studied on the same fresh platelet preparation. Drugs were added to the PRP 5 min before the addition of adrenaline. The aggregatory response was followed until the complete aggregation of the platelets. The velocity of platelet aggregation was measured as the variation of the optical transmission recorded on the chart of the aggregometer (mm per unit time).

Cell membrane preparation and binding studies

Isolated adipocytes, obtained after collagenase digestion, or platelets were washed twice in a hypotonic lysing medium composed of 2.5 mM $MgCl_2$, 1 mM $KHCO_3$, 2 mM Tris-HCl (pH 7.5) and containing ATP (0.2 mM) and several protease inhibitors: benzamidine (100 µM), phenylmethylsulphonyl fluoride (100 µM), leupeptine (1 µg ml^{-1}) and EGTA (3 mM). Crude adipocyte or platelet ghosts were pelleted by centrifugation (45,000 *g*, 15 min) at 4°C and washed twice in the lysing buffer. At the end of the washing procedure, they were resuspended in the same buffer and immediately frozen. The membrane preparation was stored at − 80°C and generally used within 1–2 days.

Binding experiments on membranes from adipocytes or platelets were carried out as previously described (Galitzky *et al.*, 1990a,b) using [^{3}H]-RX 821002 as a ligand. Briefly, thawed frozen membranes were rehomogenized and washed in 50 mM Tris-HCl, 5 mM EDTA buffer before centrifugation (40,000 *g*, 15 min at 4°C). The pellet was washed once in Tris-Mg^{2+} buffer (50 mM Tris-HCl, 0.5 mM $MgCl_2$, pH 7.5) followed by a second centrifugation. The resulting pellet was finally resuspended in buffer and immediately used. Binding studies were carried out in two different buffers; one containing 50 mM Tris-HCl, 0.5 mM $MgCl_2$, pH 7.5 and the second was supplemented with 5% bovine albumin. Incubations were conducted in a final volume of 400 µl and carried out at 25°C in a water bath for 40 min under constant shaking at 140 cycles min^{-1}. Non-specific binding was evaluated in the presence of 200 µM adrenaline. At the end ot the incubation, the reaction was stopped by the addition of 4 ml ice-cold incubation buffer followed by rapid filtration under reduced pressure through Whatmann GF/C glass fibre filters placed on a Millipore manifold. The filters were then washed twice with 10 ml of ice-cold incubation buffer, dried and placed in minivials containing 4 ml of liquid scintillation medium (Emulsifier safe, Packard INC, U.S.A.). The radioactivity retained on the filters was measured in a Packard beta counter at an efficiency of 50%. Specific binding was defined as the total binding minus the non-specific binding. In competition experiments, yohimbine, 10-OH-yohimbine and 11-OH-yohimbine were dissolved in the binding buffer, diluted and added to the assay (100 µl) just before the experiments.

Binding to plasma proteins

The binding of yohimbine, 11-OH-yohimbine and 10-OH-yohimbine to plasma proteins was evaluated by the determination of the unbound fraction obtained after ultrafiltration. The unbound fractions were obtained from pooled human plasma aliquots spiked individually with yohimbine, 11-OH-yohimbine and 10-OH-yohimbine at concentrations of 50, 100 and 200 ng ml^{-1}. Ultrafiltration was carried out in a Ultrafree-CL system, model UFC4 LTK 25, with a nominal molecular weigh cut-off of 30,000 g mol^{-1} (Millipore, Paris, France). The lack of non specific drug binding to the ultrafiltration system was checked by ultrafiltration of standard solution of yohimbine, 11-OH-yohimbine and 10-OH-yohimbine at concentrations of 50, 100 and 200 ng ml^{-1} in 0.1 M phosphate buffer saline, pH 7.4. A 2 ml aliquot of plasma or buffer was poured into the upper reservoir cup and the device was centrifuged at 25°C for 45 min at 4,000 *g* (plasma) or 5 min at 1,000 *g* (buffer). Using these conditions, 0.6 ml filtrates were obtained for both plasma and buffer.

Ultrafiltrate concentrations analysis was performed at 30°C by a high performance liquid chromatography (h.p.l.c.) method (Le Verge *et al.*, 1992), with physostigmine as internal standard. The analytical column was a Merck (Darmstadt, Germany) Lichrocart cartridge (250 mm × 4 mm) packed with Lichrosorb Si 60 (7 µm particle size) and the mobile phase was methanol, 0.02 M sodium acetate aqueous solution (95:5; v/v) at a flow rate of 1 ml min^{-1}. Fluorimetric detection was effected by a Schoeffel (Waters, Paris, France) GM 970 spectrophotofluorimeter (excitation at 280 nm, emission above 320 nm at a cell temperature of 15°C). Sample preparation: to 0.5 ml ultrafiltrate were added 5 µg ml^{-1} physostigmine chlorhydrate solution (0.1 ml), 0.5 M disodium phosphate solution pH 11 (0.25 ml) and chloroform (2 ml). The tube was shaken for 5 min and then centrifuged at 5,000 *g* for 5 min. A 1.5 ml aliquot of organic phase was evaporated under nitrogen and the residue was dissolved in 100 µl of a methanol-ethanol (85:15, v/v) mixture; 80 µl samples were injected into the chromatographic system.

Drugs and chemicals

[^{3}H]-RX 821002 (2-(2-methoxy-1,4 benzodioxan-2yl)-2-imidazoline, 43–57 Ci $mmol^{-1}$) was obtained from Amersham (England), (−)-adrenaline bitartrate and bovine albumin (fraction V) from Sigma Chemical CO. (U.S.A.), yohimbine hydrochloride from Houdé (France) and UK 14304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline) from Pfizer (U.K.). 10-OH-yohimbine and 11-OH-yohimbine (Figure 1) were

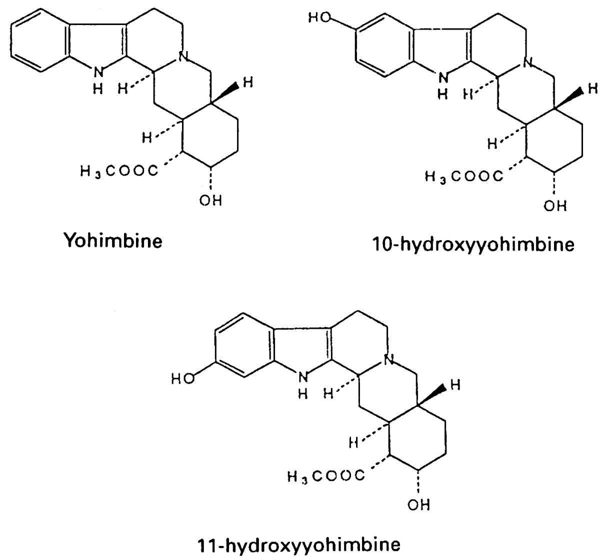

Figure 1 Structural formulae of yohimbine, 10-hydroxyyohimbine and 11-hydroxyyohimbine.

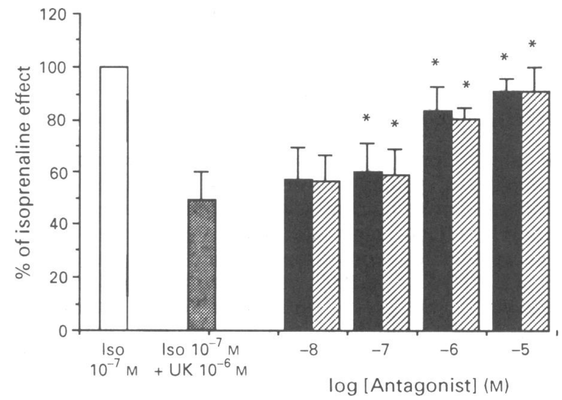

Figure 2 Effect of 11-OH-yohimbine or yohimbine on UK 14304-induced antilipolytic effects in isolated human adipocytes. Lipolysis was stimulated with 10^{-7} M isoprenaline (Iso) and in the presence of 10^{-6} M UK14304 (UK), a selective α_2-adrenoceptor agonist. The antilipolytic effect was then reversed by yohimbine (solid columns) or 11-OH-yohimbine (hatched columns). Values are expressed as percentage of the effect of spontaneous release of glycerol (i.e. basal lipolysis). Results are mean ± s.e.mean (vertical bars) of 4 separate experiments. *Significantly different when compared to isoprenaline plus UK 14304 values.

prepared by chemical synthesis from natural alkaloids and their purity was assessed by u.v. spectrometry, mass spectrometry, ^{13}C n.m.r. and ^{1}H n.m.r. (Le Verge *et al.*, 1992).

Data analysis

Values are means ± s.e.mean. Statistical analysis was made by a paired *t* test and differences were considered significant when *P* was less than 0.05.

Results

Effects of yohimbine and 11-OH-yohimbine on UK 14304-induced inhibition of lipolysis in human adipocytes

The potency of yohimbine and 11-OH-yohimbine was tested on the basis of their ability to suppress *in vitro* the inhibiting effect of 1 μM UK 14304, a selective α_2-adrenoceptor agonist inducing antilipolytic effects, on isoprenaline-induced lipolysis in human isolated adipocytes. This protocol has been described previously and offers a suitable evaluation of the antagonistic capacities of α_2-adrenoceptor antagonists (Galitzky *et al.*, 1988). Isoprenaline stimulated spontaneous lipolysis by about 254% and 1 μM UK 14304 inhibited 51% of the isoprenaline effect. The inhibitory effect of UK 14304 was significantly reduced to 40%, 14.6% and 9% in the presence of 10^{-7} M, 10^{-6} M and 10^{-5} M yohimbine or 11-OH-yohimbine respectively (Figure 2).

Effect of yohimbine and 11-OH-yohimbine on adrenaline-induced aggregation of human platelets

The aggregatory velocity of adrenaline was concentration-dependently reduced by increasing concentrations of yohimbine and 11-OH-yohimbine indicating a progressive occupation of platelet α_2-adrenoceptors (Figure 3). For 0.1 and 1 μM of each compound, the velocity of the aggregatory response to adrenaline was reduced in a concentration-dependent manner. The velocity of the adrenaline-induced aggregation was similarly reduced in the presence of yohimbine and 11-OH-yohimbine (2.1 and 2.3 fold with 0.1 μM and 3.1 and 3.4 fold with 1 μM of yohimbine or 11-OH-yohimbine respectively). For the higher concentration

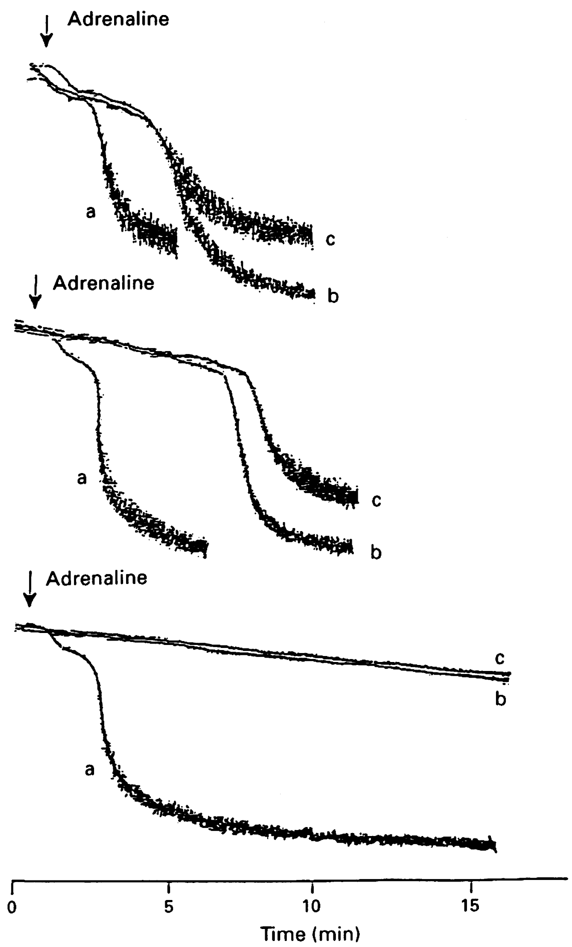

Figure 3 An example of the effect of adrenaline alone (6 μM) (a) or in the presence of various concentrations (0.1, 1 and 10 μM in the upper, central and lower panels respectively) of 11-OH-yohimbine (b) or yohimbine (c) on human platelet aggregation. The antagonists were added 5 min before the addition of adrenaline (indicated by the arrows).

(10 μM) of yohimbine or 11-OH-yohimbine, the aggregatory response to adrenaline was completely blocked.

Binding of yohimbine, 10-OH-yohimbine and 11-OH-yohimbine to adipocyte and platelet membranes

The affinity for α_2-adrenoceptors of the two hydroxylated metabolites of yohimbine was tested in binding studies on membranes from human adipocytes or platelets (two cells bearing α_2-adrenoceptors accepted as prototypes of the α_2-adrenoceptor). The competition studies were performed with 2–3 nM [^{3}H]-RX 821002, a selective radiolabelled α_2-adrenoceptor antagonist suitable for the identification of human platelet of adipocyte α_2-adrenoceptors (Galitzky *et al.*, 1990a,b). The competition curves obtained with increasing concentrations (10^{-11} M to 10^{-5} M) of yohimbine or the two hydroxylated metabolites of yohimbine on adipocyte membranes are shown in Figure 4 and the K_i values (obtained on adipocyte and platelet membranes) after computerized analysis of the curves are shown in Table 1. Yohimbine and its two hydroxylated metabolites impaired [^{3}H]-RX 821002 binding. The K_i values were found to be not significantly different in platelet or adipocyte membranes and the following order of potency was found when binding was realised in a buffer deprived of albumin: yohimbine > 11-OH-yohimbine > 10-OH-yohimbine. In buffer containing 5% albumin, the competition curve of yohimbine was shifted in a log order to the right whereas that of 11-OH-yohimbine and 10-OH-yohimbine were not significantly modified. As shown in Table 1, in the presence of albumin, K_i values for yohimbine were significantly different from those obtained in buffer supplemented with albumin.

Binding of yohimbine, 10-OH-yohimbine and 11-OH-yohimbine to plasma proteins

The concentrations of yohimbine and its two metabolites found in ultrafiltrate from plasma or buffer are given in Table 2. In the range of concentration of yohimbine used (50, 100 and 200 ng ml^{-1}, similar to that usually found in human plasma after oral yohimbine administration), the proportions of each drug bound on plasma proteins were 80.8 to 84%, 22.4 to 39.2% and 39.6 to 46.4% for yohimbine, 10-OH-yohimbine and 11-OH-yohimbine respectively. When buffer was used, the ultrafiltrate concentrations were similar for each drug to the initial added concentrations.

Discussion

The aim of the study was to define the α_2-adrenoceptor blocking potency of the two metabolites of yohimbine in man. Previous results showed that yohimbine has at least two metabolites (Figure 1) that have been identified as hydroxylated metabolites at the C-10 and C-11 positions (Le Verge *et al.*, 1992). One of these metabolites (11-OH-yohimbine) is largely present in the plasma after oral administration of

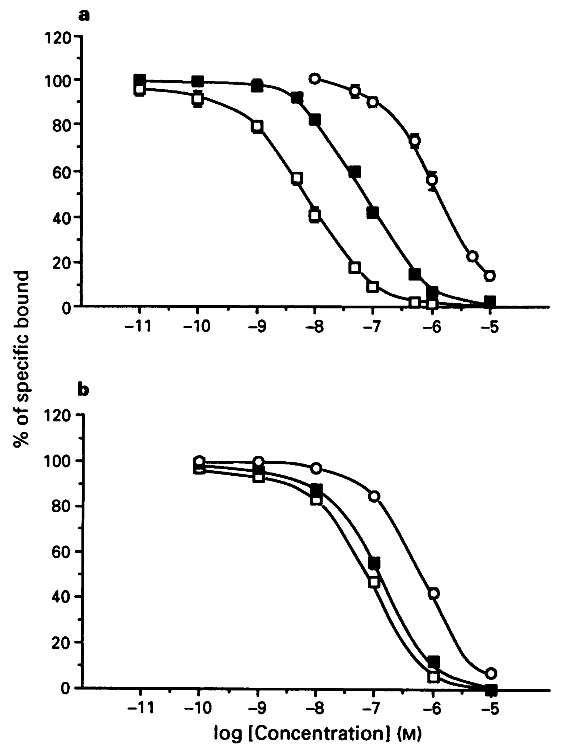

Figure 4 Inhibition of [^{3}H]-RX821002 binding on adipocyte membranes by yohimbine (□), 11-OH-yohimbine (■) and 10-OH-yohimbine (○). Competitions were performed in buffer (50 mM Tris-HCl, 0.5 mM $MgCl_2$) without (a) or containing 5% albumin buffer (b) in presence of 2–3 nM [^{3}H]-RX821002. Concentration curves reported are means of 5 experiments.

Table 2 Determination of yohimbine, 10-OH-yohimbine and 11-OH-yohimbine binding to plasma proteins

Plasma concentrations (ng ml^{-1})	50	100	200
	Filtrate	*concentrations*	(ng ml^{-1})
Yohimbine	9.6	17.5	32.1
10-OH-yohimbine	38.8	66.4	121.7
11-OH-yohimbine	30.2	55.9	107.0
Buffer concentrations (ng ml^{-1})	50	100	200
	Filtrate	*concentrations*	(ng ml^{-1})
Yohimbine	50.1	104.7	194.4
11-OH-yohimbine	45.5	94.7	193.9
10-OH-yohimbine	46.4	103.5	192.5

Values are the concentrations in the ultrafiltrate. The unbound fraction was evaluated by the difference between plasma (or buffer) and ultrafiltrate concentration.

Table 1 Inhibition constants of yohimbine, 11-OH-yohimbine and 10-OH-yohimbine on [^{3}H]-RX 821002 binding to human platelet or adipocyte membranes in the absence or presence of albumin

	Platelets; K_i (nM)		*Adipocytes;* K_i (nM)	
	(–)	+ *Albumin*	(–)	+ *Albumin*
Yohimbine	1.65 ± 0.11	15.6 ± 1.82*	2.74 ± 0.67	25.3 ± 2.1*
11-OH-yohimbine	25.1 ± 2.3	28.3 ± 4.7	26.6 ± 3.1	32.6 ± 4.3
10-OH-yohimbine	267 ± 62	241 ± 47	313 ± 19	278 ± 17

Competition experiments were performed in buffer (50 mM Tris-HCl, 0.5 mM $MgCl_2$) deprived (–) or containing 5% albumin in the presence of 2–3 nM [^{3}H]-RX821002. The antagonist inhibition data were analysed using INHIBITION, a computerized programme for curve fitting to a one-site inhibition model (Barlow, 1983). Inhibition constants (K_i) were calculated from the equation: $K_i = EC_{50}/(1 + [[^3H]\text{-RX821002}]/K_D)$, where EC_{50} is the concentration of drug displacing 50% of the bound radioligand and K_D the affinity of [^{3}H]-RX821002 determined in saturation experiments. The values reported are means ± s.e.mean of 4–5 determinations. *Significantly different when compared to values obtained in albumin-free medium.

yohimbine and exhibits a longer elimination half-life than the parent drug (6 h versus 1 h, respectively). This supports the hypothesis of a first pass effect and consequently low oral bioavailability of yohimbine in man.

The present study demonstrates that 10-OH-yohimbine or 11-OH-yohimbine have affinity for α_2-adrenoceptors since they displaced [^{3}H]-RX 821002 binding on human adipocyte and platelet membranes. However, the order of potency found (yohimbine > 11-OH-yohimbine > 10-OH-yohimbine) and the calculated K_i (Table 1) require some comment. The affinity of the 11-OH-yohimbine derivative for adipocyte or platelet α_2-adrenoceptors is within the range currently found for various α_2-adrenoceptor antagonists (Lafontan *et al.*, 1992) which induce various pharmacodynamic effects (increase in plasma noradrenaline and non-esterified fatty acid levels). However, 10-OH-yohimbine does not exhibit an affinity compatible with putative pharmacodynamic effects. This point is supported by the lack of pharmacodynamic effects of nicergoline or SKF 104078, two α_2-adrenoceptor antagonists exhibiting a low affinity (K_i> 90 nM) for α_2-adrenoceptors (Lafontan *et al.*, 1992). The interest of 11-OH-yohimbine is that after oral administration of yohimbine in man its concentration is 10 fold higher than that of the parent drug (Le Verge *et al.*, 1992).

Biological measurements of the α_2-adrenoceptor antagonist properties of 11-OH-yohimbine revealed that, whatever the cell model used (human platelets or adipocytes), this compound suppressed, in a concentration-dependent manner, the effect of the stimulation of the α_2-adrenoceptors (i.e. antagonism of the UK 14304-induced antilipolysis in adipocytes or the adrenaline-induced aggregation in platelets). Surprisingly, its potency in suppressing the agonist-induced effect is quite similar to that of yohimbine. This discrepancy between affinities for α_2-adrenoceptors (evaluated in binding studies carried out in medium deprived of proteins) and the α_2-adrenoceptor blocking capacities of yohimbine and 11-OH-yohimbine (evaluated in biological studies carried out in plasma or in medium supplemented with albumin) may be explained by a difference in the protein binding of the substances. The plasma protein binding study of yohimbine, 10-OH-yohimbine and 11-OH-yohimbine showed that yohimbine exhibited a higher affinity for proteins than its two metabolites (in the range of 50 to 200 ng per ml plasma which corresponds to usual concentrations found after oral yohimbine administrations; Le Verge *et al.*, 1992; Berlan *et al.*, 1991). So, in biological studies, the weaker affinity of 11-OH-yohimbine for α_2-adrenoceptors could be counterbalanced by the 3–4 fold higher concentration of its free form. This is supported by binding studies carried out in medium supplemented with protein (albumin) where the apparent affinities (K_i, Table 1) of yohimbine and 11-OH-yohimbine for α_2-adrenoceptors became approximately equivalent.

In addition, yohimbine is a highly lipid-soluble compound which enters the brain (Lambert *et al.*, 1978; Brannan *et al.*, 1991). The 11-hydroxylated metabolite is a less lipid-soluble compound eliminated through renal excretion (Le Verge *et al.*, 1992) and it is likely that this product may have a lower capacity to enter the brain. This compound may be useful (in comparison with yohimbine) in evaluating the relative part of the peripheral and central α_2-adrenoceptors involved in the pharmacodynamic effects of α_2-adrenoceptor antagonists.

The results presented here show that the two hydroxylated metabolites of yohimbine exhibit α_2-adrenoceptor antagonist properties. The main metabolite found in plasma after oral administration in man, 11-OH-yohimbine, has higher affinity for α_2-adrenoceptors in binding studies than the 10-OH-metabolite and is able to suppress the functional effects induced by α_2-adrenoceptor stimulation with the same efficacy as yohimbine in plasma or in medium containing protein. In conclusion, these results give a better understanding of the pharmacodynamic effects of yohimbine in man because the effects of yohimbine are frequently found to be longer lasting than those predicted by pharmacokinetic data and may indicate a measure of peripheral selectivity at later time intervals. This could be also a relevant explanation for its lack of efficacy in some cases correlated with its variable oxidative metabolism. For this purpose a complete pharmacokinetic study of yohimbine and its metabolites is to be undertaken.

References

BARLOW, R.B. (1983). *Biodata Handling with Microcomputers.* Amsterdam: Elsevier Science publishers.

BERLAN, M., GALITZKY, J., RIVIERE, D., FOUREAU, M., TRAN, M.A., FLORES, R., LOUVET, J.P., HOUIN, G. & LAFONTAN, M. (1991). Plasma catecholamine levels and lipid mobilization induced by yohimbine in obese and non-obese women. *Int. J. Obesity*, **15**, 305–315.

BERLIN, I., CRESPO-LAUMONNIER, B., TURPIN, G. & PUECH, A.J. (1989). The alpha-2 adrenoceptor antagonist yohimbine does not facilitate weight loss but blocks adrenaline-induced platelet aggregation in obese subjects. *Therapie*, **44**, 301.

BRANNAN, T., MARTINEZ-TICA, J. & YAHR, M.D. (1991). Effect of yohimbine on brain monoamines: an in vivo study. *J. Neural. Transm.*, **3**, 81–87.

CHARNEY, D.S., PRICE, L.H. & HENINGER, G.R. (1986). Desipramine-yohimbine combination treatment of refractory depression. *Arch. Gen. Psychiatry.*, **43**, 1155–1161.

GALITZKY, J., LARROUY, D., BERLAN, M. & LAFONTAN, M. (1990a). New tools for human fat cell alpha-2 A-adrenoceptor characterization. Identification on membranes and on intact cells using the new antagonist [^{3}H]-RX 821002. *J. Pharmacol. Exp. Ther.*, **252**, 312–319.

GALITZKY, J., SENARD, J.M., LAFONTAN, M., STILLINGS, M., MONTASTRUC, J.L. & BERLAN, M. (1990b). Identification of human platelet α_2-adrenoceptors with a new antagonist [^{3}H]-RX 821002, a 2-methoxy derivative of idazoxan. *Br. J. Pharmacol.*, **100**, 862–866.

GALITZKY, J., TAOUIS, M., BERLAN, M., RIVIERE, D., GARRIGUES, M. & LAFONTAN, M. (1988). Alpha2-antagonist compounds and lipid mobilization: evidence for a lipid mobilizing effect of oral yohimbine in healthy male volunteers. *Eur. J. Clin. Invest.*, **18**, 587–594.

GOLDBERG, M.R., HOLLISTER, A.S. & ROBERTSON, D. (1983). Influence of yohimbine on blood pressure, autonomic reflexes and plasma catecholamines in humans. *Hypertension*, **5**, 772–778.

GOLDBERG, M.R. & ROBERTSON, D. (1983). Yohimbine: a pharmacological probe for study of the alpha$_2$-adrenoceptors. *Pharmacol. Rev.*, **35**, 143–180.

GRUNHAUS, L., TIONGCO, D., ZELNIK, T., FLEGEL, P., HOLLINGSWORTH, P.M. & SMITH, C.B. (1989). Intravenous yohimbine: selective enhancer of norepinephrine and cortisol secretion and systolic blood pressure in humans. *Clin. Neuropharmacol.*, **12**, 106–114.

GUTHRIE, S.K., HARIHARAN, M. & GRUNHAUS, L. (1990). Yohimbine bioavailability in humans. *Eur. J. Clin. Pharmacol.*, **39**, 409–411.

LAFONTAN, M., BERLAN, M., GALITZKY, J. & MONTRASTRUC, J.L. (1992). Alpha2 adrenoceptors in lipolysis: alpha$_2$ antagonists and lipid mobilizing strategies. *Am. J Clin. Nutr.*, **55**, S219–S227.

LAMBERT, G.A., LANG, W.J., FRIEDMAN, E., MELLER, E. & GERSHON, S. (1978). Pharmacological and biochemical properties of isometric yohimbine alkaloids. *Eur. J. Pharmacol.*, **49**, 39–48.

LE VERGE, R., LE CORRE, P., CHEVANNE, F., DÖE DE MAINDREVILLE, M., ROYER, D. & LEVY, J. (1992). Determination of yohimbine and its two hydroxylated metabolites in humans by high-performance liquid chromatography and mass spectral analysis. *J. Chromatogr.*, **574**, 283–292.

MAURIEGE, P., GALITZKY, J., BERLAN, M. & LAFONTAN, M. (1987). Heterogenous distribution of beta- and alpha$_2$-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. *Eur. J. Clin. Invest.*, **17**, 156–165.

NICHOLS, A.J. & RUFFOLO, R.R. (1991). Functions mediated by α-adrenoceptors. In *α-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology*. ed. Ruffolo, R.R. *Prog. Basic. Clin. Pharmacol.*. Vol. 8. pp. 115–179: Basel: Kager.

ONROT, J., GOLDBERG, M.R., BIAGGIONI, I., WILEY, R.G., HOLLISTER, A.S. & ROBERTSON, D. (1987). Oral yohimbine in autonomic failure. *Neurology*, **37**, 215–220.

OWEN, J.A., NAKATSU, S.L., FENEMORE, J., CONDRA, M., SURRIDGE, D.H.C. & MORALES, A. (1987). The pharmacokinetics of yohimbine in man. *Eur. J. Pharmacol.*, **32**, 577–582.

PESKIND, E.R., VEITH, R.C., DORSA, D.M., GRUMBRECHT, G. & RASKING, M.A. (1989). Yohimbine increases cerebrospinal fluid and plasma norepinephrine but not arginine vasopressine in humans. *Neuroendocrinol.*, **50**, 286–291.

RISPAIL, Y., SCHMITT, L., BERLAN, M., MONTASTRUC, J.L. & MONTASTRUC, P. (1990). Yohimbine increases salivary secretion in depressed patients treated by tricyclic antidepressants. *Eur. J. Clin. Pharmacol.*, **39**, 425–426.

VILLENEUVE, A., BERLAN, M., LAFONTAN, M., CARANOBE, C., BONEU, B., RASCOL, A. & MONTASTRUC, J.L. (1985). Platelet alpha$_2$-adrenoceptors in Parkinson's disease: decrease number in untreated patients and recovery after treatment. *Eur. J. Clin. Invest.*, **15**, 403–407.

WIELAND, O. (1957). Eine enzymatische Methode zur Bestimmung von Glycerin. *Biochem. Z.*, **239**, 313–319.

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